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ORIGINAL PAPER

Characterization of silver nanoparticle produced by *Pseudopediastrum boryanum* (Turpin) E. Hegewald and its antimicrobial effects on some pathogens

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Abstract

The aim of this research was to investigate the antimicrobial activity of silver nanoparticles (AgNPs) biosynthesized by *Pseudopediastrum boryanum* (Turpin) E. on several human pathogen microorganisms. AgNPs were isolated from *P. boryanum*. The biosynthesis of AgNPs was carried out using a UV–visible spectrophotometer and FTIR spectroscopy analysis. The antimicrobial activity of AgNPs was evaluated against various pathogen microorganisms using the well diffusion method, and MIC was estimated by qualitative experimentation by microbroth dilution method. The antimicrobial activities of AgNPs at three different concentrations (1 mM, 2 mM and 3 mM) were measured using the diameter of the inhibition zone (DIZ) of the pathogen microorganisms. AgNPs demonstrated various antimicrobial effects on pathogen microorganisms at different concentrations. The highest antimicrobial activity was determined in *Proteus vulgaris* [DIZ = 30 ± 0.2 mm (2 and 3 mM)], followed by *Candida parapsilosis* (M006) [DIZ = 25 ± 0.1 mm (3 mM)], followed by *Pseudomonas aeruginosa* [DIZ = 20 mm (2 and 3 mM)] and *Candida parapsilosis* (M006) [DIZ = 20 mm (1 and 2 mM)]. The lowest antibacterial effects of AgNPs were observed on *Aeromonas hydrophila* [DIZ = 5 ± 0.1 mm (3 mM)], *Staphylococcus epidermidis* [DIZ = 5 ± 0.1 mm (1 mM)], *Candida parapsilosis* [DIZ = 5 ± 0.1 mm (2 mM)] and *Candida albicans* [DIZ = 5 ± 0.1 mm (3 mM)]. Gram-negative bacteria *Proteus mirabilis*, *Enterobacter aerogenes, Salmonella typhimurium, Shigella dysenteriae, Escherichia coli* and *Serratia marcescens* and Gram-positive bacteria *Listeria monocytogenes* and *Enterococcus faecalis* exhibited no zone of inhibition. Our results confirm that AgNPs biosynthesized from *P. boryanum* may be used as an effective antimicrobial agent against human pathogens.

Keywords *Pseudopediastrum boryanum* · Biosynthesis · Microalgae · Silver nanoparticles · Antimicrobial activity

Introduction

Nanotechnology has many potential technological applications spanning various areas of science. In general, it may be described as the formation, synthesis and application of convenient materials and structures through control of matter at the nanometer scale (1–100 nm) (Mansoori et al. 2007). With this technology, the surface-to-volume ratios of the materials used increase in nanodimensions, and changes in the properties of the material used can be achieved (Ravichandran 2010; Khan et al. 2017; Ranjitha and Rai 2017). Although nanoparticles are widely used in the electronics, food technology (fishery and aquaculture) and energy sectors, studies of their medical use have also been very promising (Simonin and Richaume 2015). In particular, it has recently been recognized that metal nanoparticles have high antimicrobial (antiviral, antibacterial and antifungal)

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properties (Schrofel et al. 2014). Silver nanoparticles (AgNPs) have attracted considerable interest among numerous metal nanoparticles, such as ZrO_2, Al_2O_3, CeO_2, Fe_3O_4 and MgO (Ravikumar et al. 2012) in this context (Gong et al. 2007). Silver is known bactericides and is thought to be non-toxic at low concentrations.

As the need for the metal nanoparticles in industry has increased, various problems have emerged with the classic technological methods used for their production. AgNP synthesis most commonly involves the chemical reduction of silver salts with sodium borohydride or sodium citrate (Simonin and Richaume 2015). However, this type of production is expensive, the resulting particles have high toxic contents, and particle stability is reported to be low (Narayanan and Sakthivel 2010). Various methods have been investigated in order to overcome these problems. In this context, the biosynthesis of metal nanoparticles from several microorganisms has been reported as a highly promising technology for producing effective metal nanoparticles in large quantities, while avoiding environmental toxicity and biological hazards. As a green and environment-friendly method, several bacteria, yeasts and fungi have been shown to be capable of synthesizing and producing effective metal nanoparticles (Duncan 2011). Nanoparticles can be produced at lower cost in large quantities using biological synthesis compared to other production techniques (Hulkoti and Taranath 2014; Pabba et al. 2013). The newest materials among the environment-friendly methods used for the production of metal nanoparticles are green microalgae. Research on nanoparticle synthesis from metals from microalgae has become interest in biotechnology only recently and nanoparticle synthesis has been carried out from gold (Luangpipat et al. 2011), palladium (Lengke et al. 2007) and nickel (Gong et al. 2007) so far. Several metals, metalloids and metallic nanoparticles affect microalgae growth and metabolism (Miazek et al. 2015). El-Sheekh and El-Kassas (2016) reported that AgNPs and AuNPs biosynthesized from algae demonstrated exceptional antimicrobial and cytotoxic effects. Most algae used in the biosynthesis of AgNPs to date have been marine species (Ramakritinan et al. 2013; Sahayaraja et al. 2012; Merin et al. 2010). The antibiological effects of AgNPs biosynthesis from species belonging to Cyanobacteria and Chlorophyta have also been tested (Patel et al. 2015). The biosynthesis and antimicrobial potential of AgNPs produced using the green microalgae *Chlamydomonas reinhardtii* have been studied in *Listeria monocytogenes*, a pathogenic bacterium, and have been shown to inhibit pathogen growth (Ahmadi et al. 2016).

Pseudopediastrum boryanum (Turpin) E. Hegewald is colony-like green algae that occur naturally in stagnant freshwater bodies of the Chlorophyceae class. A member of the genus *Pseudopediastrum*, the species is widely distributed worldwide (Komarek and Jankovska 2001). The algal genus Pediastrum, particularly the species *Pseudopediastrum boryanum*, has attracted the interest of researchers in the context of wastewater purification due to its rapid reproductive capability (Park et al. 2014). Sigee et al. (2002) studied the *Pediastrum duplex* to determine the molecular characterization of microalgae using FTIR spectroscopy. *Pediastrum duplex* was selected as the organism to be analyzed because it has a FTIR spectrum with clear bands and is an important component of microalgae in freshwater. To the best of our knowledge, *P. boryanum* has not previously been studied for its potential in metal nanoparticle production. In this study, we report the synthesis of AgNPs using *P. boryanum* microalgae and also assess its antimicrobial activity against 24 common human pathogenic microorganisms.

Materials and methods

Microalgae culture and growth conditions

Pseudopediastrum boryanum was isolated from samples collected from various freshwater deposits in Ankara (Turkey). The one-cell growth technique was used for strain isolation (Parvin et al. 2007). Molecular characterization of *P. boryanum* strains was performed using Fourier transform infrared (FTIR) spectroscopy and polymerase chain reaction (PCR). This strain is conserved in the Ahi Evran University of Culture Collections of Algae (AEU-CCA) and is encoded as *CCA02Pdr01*. Cultures were cultivated in BG 11 medium in the form of 270 ml of medium + 30 ml of suspension culture. The light source (Philips cool daylight, 50 µmol m^{-2} s^{-1}) was applied horizontally at a distance of 22 cm from the cultures with a period of 16 L:8 D, and cultivation was performed at 22–25 °C. The pH value of the medium was adjusted to 6.8.

In previous studies, the optical density of *P. boryanum* was determined at 670 nm during the production of BG 11 medium under culture conditions. Using growth kinetics, specific growth rates and duplication times were calculated by Godoy-Hernández and Vázquez-Flota (2006). In BG 11 medium, the doubling time was 0.0577 day^{-1} and the specific growth rate was 0.6021 day^{-1}. The cell density was 2.19×10^6 cells/mL in BG-11 medium at the end of 11th day (Duygu et al. 2018).

Test microorganisms

Twenty-four human pathogen microorganisms (18 bacteria and 6 yeasts) were used in this study, as shown in Table 1. Nutrient broth was used for grown these cultures and incubated at 30 ± 1 °C overnight.

Table 1 Test organisms

Human pathogen microorganisms (bacteria and yeasts)	Code	Type
Proteus mirabilis	ATCC 29906	Gram-negative
Pseudomonas aeruginosa	ATCC 27853	Gram-negative
Enterobacter aerogenes	ATCC 51342	Gram-negative
Salmonella typhimurium	ATCC 14028	Gram-negative
Shigella dysenteriae	ATCC 11835	Gram-negative
Escherichia coli	ATCC 25924	Gram-negative
Escherichia coli	ATCC 25922	Gram-negative
Aeromonas hydrophila	ATCC 7966	Gram-negative
Klebsiella pneumoniae	ATCC 21541	Gram-negative
Serratia marcescens	ATCC 13880	Gram-negative
Vibrio anguillarum	ATCC 43312	Gram-negative
Proteus vulgaris	ATCC 29905	Gram-negative
Bacillus cereus	709 Roma	Gram-positive
Listeria monocytogenes	ATCC 35152	Gram-positive
Staphylococcus aureus	ATCC 29213	Gram-positive
Enterococcus faecalis	ATCC 29212	Gram-positive
Bacillus subtilis	ATCC 10774	Gram-positive
Staphylococcus epidermidis	ATCC 12228	Gram-positive
Saccharomyces boulardii	ATCC MYA-796	Yeast
Candida albicans	ATCC 10231	Yeast
Candida tropicalis	M007	Yeast
Candida parapsilosis	M006	Yeast
Candida parapsilosis	ATCC 22019	Yeast
Candida glabrata	ATCC 15126	Yeast

Synthesis of AgNPs by algal culture

Microalgae *P. boryanum* were harvested by centrifugation at 3000 rpm for 15 min and were washed three times with sterile distilled water. One gram of each wet weight biomass of culture was then suspended in 20 ml of 1 mM, 2 mM and 3 mM aqueous $AgNO_3$. Fresh BG 11 medium with the addition of $AgNO_3$ was used as a control. Both sets of cultures were incubated at 25 ± 1 °C, under fluorescent light (Philips cool daylight, 50 µmol m^{-2} s^{-1}) for 72 h. Samples were taken at different time intervals (24, 48 and 72 h). This experiment was repeated three times, and the data obtained were found to be consistent for the tested strains.

Characterization of AgNPs

The biosynthesis of AgNPs was confirmed by the change in color of the $AgNO_3$ solution. AgNPs were examined visually for a change from light brown to dark brown in the color of the culture medium. Absorbance spectra were measured throughout 72 h. One milliliter aliquot samples were collected, and the absorbance of the UV–Vis spectra at between 200 and 800 nm was measured by using a spectrophotometer (Thermo Scientific Spectrophotometer Genesys 10S). UV–Vis measurements were taken at 24-, 48- and 72-h intervals.

Fourier transform infrared (FTIR) measurements

Fourier transform infrared (FTIR) spectral analysis was used to detect biomolecules responsible for reducing Ag^+ ions. Infrared analysis was performed at the Ahi Evran University Central Laboratory, Kırşehir, Turkey, using a Thermo Scientific Nicolet 6700 model FTIR spectrometer. Five milliliters of sample with AgNPs was taken and washed three times with distilled water. Samples were dried under vacuum at 40 °C and analyzed using the FTIR instrument. The spectrum was recorded in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}.

Determination of antimicrobial activity (disk diffusion test)

The antimicrobial activity of AgNPs synthesized by *P. boryanum* was tested against pathogen microorganisms (18 bacteria and 6 yeasts) strains using the agar well diffusion method. Pathogen microorganisms were also produced in Trypticase soy broth, and each strain was cultivated separately with Trypticase soy agar using sterile cotton swabs. All test microorganisms (approximately 10^8 cells/mL) were produced over 24 h at different AgNP concentrations (0.01–5 µg/mL). Following this incubation, the viability of bacterial cells was determined by CFU counting. Wells with a diameter of 6 mm were prepared in the Trypticase soy agar plates using sterile gel puncture. After incubation at 37 °C for 48 h, the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (DIZ). Distilled water is used as negative control, while commercial antibiotic disks (ampicillin 10 mcg, gentamicin 10 mcg and nystatin 50 mcg) are used as positive control.

Determination of minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations (MICs) were determined in 96-well microtitre plates. All isolates were grown in nutrient broth at 37 °C overnight; then, the bacterial cultures were added into 96-well plates containing diluted samples of AgNPs (10–100 µl). Each sample was tested in triplicate, and the results were recorded after 24-h incubation periods (Table 3).

Antimicrobial activity index

The antimicrobial activity of the samples was compared with the antimicrobial index of the AgNPs separately, and the

following formula was used for the calculation (Ghasemi et al. 2003). The results are given in Table 4.

$$\text{Antimicrobial Index} = (\text{AgNPs inhibition zone}/\text{Antibiotic inhibition zone}) \times 100$$

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as means (±) ($n = 3$) plus the standard deviation of the means. Statistical analysis was performed using Microsoft Excel.

Results and discussion

To the best of our knowledge, this is the first study to investigate the antimicrobial effect of AgNPs biosynthesized from the freshwater microalgae *P. boryanum.* Our most important finding is that biologically synthesized AgNPs from *P. boryanum* exhibited excellent antimicrobial effects against several pathogen microorganisms in different concentrations.

Characterization of synthesized silver nanoparticles

The strength of this study lies in its well-planned methodology. We confirmed the biosynthesis of AgNPs from *P. boryanum* with several methods. The biosynthesis of AgNPs was confirmed not only by means of visual references, but also with a UV–visible spectrophotometer and FTIR spectroscopy analysis. In the first step, during the reaction in which AgNPs were biosynthesized from *P. boryanum,* the algal bright green color is changed to brown after 72 h; otherwise, discoloration from bright green to brown was observed when algal biomass was exposed to 1 mM, 2 mM and 3 mM silver nitrate ions, demonstrating the biosynthesis of silver nanoparticles (Fig. 1).

UV–visible spectral analysis

This color change was derived from the excitation of surface plasmon vibrations in the metal nanoparticles. This was confirmed by UV–visible spectrophotometer analysis. Formation of AgNPs in *P. boryanum* cells was followed by a change in UV–Vis absorbance peaks associated with surface plasmon resonance of the $AgNO_3$ solution. The color change to brown was due to excitation of surface plasmon vibration, indicating the formation of AgNPs (Fig. 1e). At UV–Vis spectroscopy, a surface plasmon resonance peak was observed at 417 nm after a 72-h reaction time (Fig. 2). Kong and Jang (2006) reported that the absorption spectrum of AgNPs prepared by biological reduction exhibited surface plasmon absorption between 400 and 420 nm, proving the presence of AgNPs. Marine red alga by Ramakritinan et al. (2013) detected surface plasmon absorption at 419 nm at the end of 120 h of the AgNPs obtained from *Gracilaria* sp. AgNP synthesis from the green microalgae *Chlorella vulgaris* and *Chaetoceros calcitrans*, and Karthikeyan et al. (2015) reported that the absorbance spectra of the AgNPs containing aqueous medium peaked at 420 and 436 nm, respectively.

Fourier transform infrared (FTIR) analysis

AgNPs synthesized by the green microalgae *P. boryanum* were subjected to FTIR spectrum analysis to identify whether the biomolecules constitute stabilizing and reducing agents. Typical appearances of a culture absorption spectrum are shown in (Fig. 3) and exhibit 10 clear bands over a wave number range of 4000–500 cm^{-1}. FTIR spectrum bands were assigned on the basis of standards (Sigee et al. 2002; Nauman 2002; Dean et al. 2007) and published FTIR spectra for specific molecular groups (Table 2). Band distributions were predicted as residual water (–OH; band 1), lipid (–CH2; bands 2 and 3), cellulose (–C=O; band 4), amide (protein; bands 5 and 6), nucleic acid (> P=O; bands

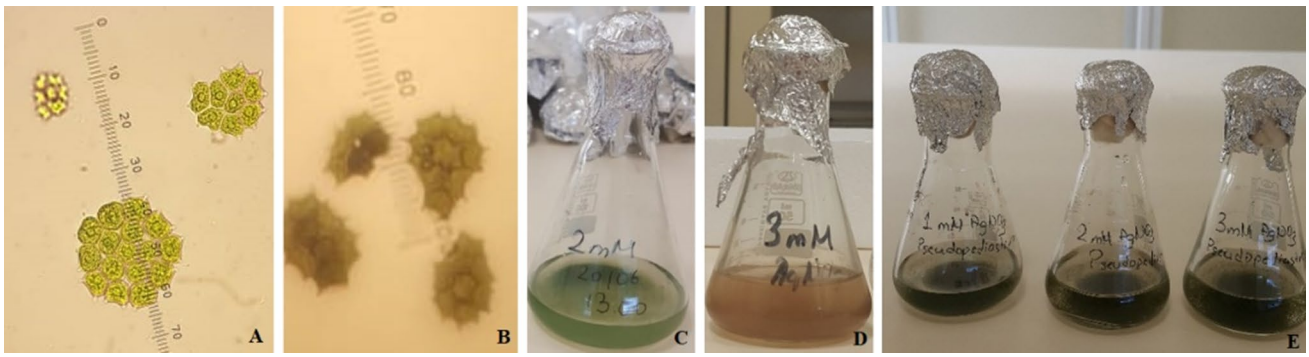

Fig. 1 **a** Microscope image of *P. boryanum*, **b** microscope image of *P. boryanum* treated with $AgNO_3$, **c** before addition of silver nitrate, **d** addition of silver nitrate, **e** after incubation period

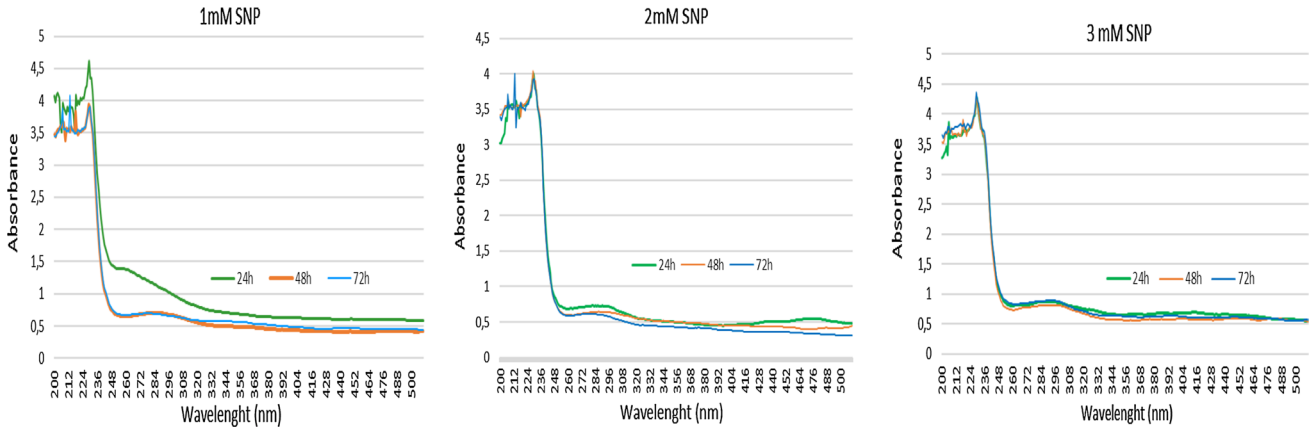

Fig. 2 UV–Vis absorption spectra of AgNPs (1 mM, 2 mM and 3 mM) prepared by the supernatant of *P. boryanum* (after 24-, 48- and 72-h incubation)

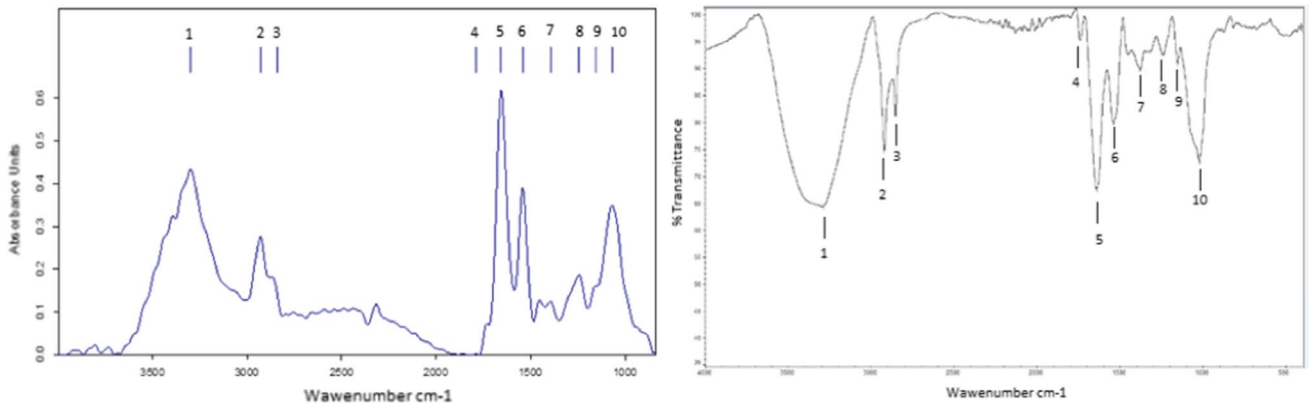

Fig. 3 FTIR spectra of microalgae *P. boryanum* (left) and FTIR spectra of AgNPs prepared by the supernatant of *P. boryanum* (right)

8 and 10) and starch (–C–O; bands 9 and 10). The region between 3100 and 2800 cm^{-1} exhibits the C–H stretching vibrations of $–CH_3$ and $>CH_2$ functional groups. The region between 1800 and 1500 cm^{-1} is dominated by the conformation amide I and amide II bands for nearly all microorganisms (Nauman 2002). The band at 3289 cm^{-1} corresponds to protein *ν*(N–H) stretching (amide A). Protein spectra were characterized by two strong features at 1639 cm^{-1} (amide I) and 1536 cm^{-1} (amide II). These bands were primarily due to C=O stretching vibration and a combination of N–H bending and C–N stretching vibrations in amide complexes, respectively (Duygu et al. 2012). Lipid spectra were characterized by two sets of strong vibrations, C–H at 2919 cm^{-1} and the C=O mode of the side chain from the ester carbonyl group at 1724 cm^{-1}, starch absorption bands due to C–O–C of polysaccharides at 1149 cm^{-1} and 1019 cm^{-1}. The peaks appearing in the region 1639 cm^{-1} are attributed to the stretching vibration of the *ν*(C=O) group that is characteristic of proteins shifted after synthesis of AgNPs. Proteins can play an important role in the formation and stabilization of nanoparticles because they bind to nanoparticles through cysteine residues or free amino groups (Jeevan et al. 2012). This finding shows that the protein molecules may be involved in AgNP formation and subsequent stabilization. Tentative assignments of bands observed in FTIR spectra of *P. boryanum* and AgNPs biosynthesized using *P. boryanum* are presented in Table 2 and Fig. 3.

Antimicrobial activity of AgNPs

In this research, antimicrobial activity of AgNPs synthesized by *P. boryanum* strains was tested against several Gram-negative and Gram-positive bacteria and yeast strains (Table 1) using standard DIZ measurement. The mean values of three replicates of the DIZ (in millimeters) around each well with AgNP solution are shown in Table 3. AgNPs (1 mM, 2 mM and 3 mM) were prepared at three different concentrations during investigation of antimicrobial effects. These different concentrations of AgNPs exhibited varying antimicrobial effects on pathogen microorganisms.

Table 2 Tentative assignment of bands found in FTIR spectra of *P. boryanum* and biosynthesized AgNPs using *P. boryanum*

Band number	Main peak (cm^{-1}) $AgNO_3$ + *P. boryanum* (*P. boryanum* in brackets)	Typical band assignment from the literature	Wavenumber range (cm^{-1})
1	3289 (3299)	*Water* ν(O–H) stretching *Protein* ν(N–H) stretching (amide A)	3646–3026
2	2919 (2929)	*Lipid* $\nu_{as}(CH_2)$ stretching of methylene	2943–2902
3	2851 (2817)	*Lipid* $\nu_{s}(CH_2)$ stretching of methylene	2864–2837
4	1724 (1787)	*Cellulose* *Fatty acids* ν(C=O) stretching of esters	1763–1712
5	1639 (1656)	*Protein* Amide I band Mainly ν(C=O) stretching	1709–1587
6	1536 (1542)	*Protein* Amide II band Mainly δ(N–H) bending and ν(C–H) stretching DNA Double-bound vibrations of bases	1577–1481
7	1377 (1394)	*Lipid* $\delta_{s}(N(CH_3)_3)$ bending of methyl *Protein* $\delta_{s}(CH_2)$ and $\delta_{s}(CH_3)$ bending of methyl	1408–1358
8	1238 (1243)	*Nucleic acid* $\nu_{as}(>P=O)$ stretching of phosphodiesters	1294–1194
9	1149 (1149)	*Starch* ν(C–O) stretching and complex sugar ring modes	1196–1136
10	1019 (1069)	*Starch* ν(C–O) stretching and complex sugar ring modes *Nucleic acid* $\nu_{s}(>P=O)$ stretching of phosphodiesters	1136–980

Band assignment based on Sigee et al. (2002), Nauman (2002) and Dean et al. (2007)

The highest antimicrobial activity was measured against *P. vulgaris* [DIZ = 30 ± 0.2 mm (2 and 3 mM)], followed by *P. parapsilosis* (M006) [DIZ = 25 ± 0.1 mm (3 mM)] and *P. aeruginosa* [DIZ = 20 ± 0.1 mm (2 and 3 mM)]. The lowest antibacterial effect of AgNPs was observed against *A. hydrophila* [DIZ = 5 ± 0.1 mm (3 mM)], *S. epidermidis* [DIZ = 5 ± 0.1 mm (1 mM)], *C. parapsilosis* (ATCC 22019) [DIZ = 5 ± 0.1 mm (2 mM)] and *C. albicans* [DIZ = 5 ± 0.1 mm (3 mM)]. The Gram-negative bacteria *P. mirabilis*, *E. aerogenes, S. typhimurium, S. dysenteriae, E. coli, S. marcescens* and the Gram-positive bacteria *L. monocytogenes* and *E. faecalis* exhibited no zone of inhibition. The inhibition of AgNPs synthesized by *P. boryanum* against some pathogen microorganisms in their agar cultures is presented in Fig. 4. The antibacterial effect of AgNPs was effective at different concentrations in 6 out of 12 Gram-negative bacterial strains, 4 out of 6 Gram-positive bacterial strains and in all 6 yeasts.

Our findings revealed that AgNPs obtained from *P. boryanum* exhibit various antimicrobiological effects against microorganisms in different concentrations (1 mM, 2 mM and 3 mM). Several microorganisms such as *E. aerogenes, S. typhimurium, S. dysenteriae, E. coli* (both), *S. marcescens, L. monocytogenes* and *E. faecalis* were resistant to AgNPs in any concentrations. We also observed that the antimicrobiological effects of AgNPs increased in higher concentrations. At a 1 mM AgNP concentration, 7 microorganisms out of 25 exhibited susceptibility (in terms of DIZ), compared to 12 out of 25 at 2 mM, and 15 out of 25 at 3 mM. AgNPs demonstrated antimicrobial effects against *S. aureus, S. epidermidis, C. tropicalis, C. parapsilosis and C. glabrata* at all three concentrations (1 mM, 2 mM and 3 mM). Antimicrobial effects of AgNPs were also observed in *P. aeruginosa*, *V. anguillarum, P. vulgaris, B. cereus, S. boulardii* and *C. parapsilosis* at 2 mM and 3 mM concentrations. However, antimicrobiological effects of AgNPs were only

Table 3 Diameter of inhibition zone (DIZ) and MIC of the different concentrations of AgNPs biosynthesized from *P. boryanum*

Human pathogen microorganisms	1 mM AgNP DIZ (mm)	2 mM AgNP DIZ (mm)	3 mM AgNP DIZ (mm)	$AgN0_3$ + *P. boryanum* MIC (µg/ml)	Antibiotic positive control		
					AMP[a]	GEN[b]	NS[c]
P. mirabilis (ATCC 29906)	NCD	NCD	NCD	NCD	16	21	0
P. aeruginosa (ATCC 27853)	NCD	20 ± 0.1	20 ± 0.1	80	25	26	0
E. aerogenes (ATCC 51342)	NCD	NCD	NCD	NCD	15	17	0
S. typhimurium (ATCC 14028)	NCD	NCD	NCD	NCD	14	15	0
S. dysenteriae (ATCC 11835)	NCD	NCD	NCD	NCD	12	12	0
E. coli (ATCC 25924)	NCD	NCD	NCD	NCD	10	20	0
E. coli (ATCC 25922)	NCD	NCD	NCD	NCD	10	20	0
A. hydrophila (ATCC 7966)	NCD	NCD	5 ± 0.1	20	0	20	0
K. pneumoniae (ATCC 21541)	NCD	NCD	17 ± 0.1	60	25	27	0
S. marcescens (ATCC 13880)	NCD	NCD	NCD	NCD	14	16	0
V. anguillarum (ATCC 43312)	NCD	12 ± 0.1	15 ± 0.1	40	20	22	0
P. vulgaris (ATCC 29905)	NCD	30 ± 0.2	30 ± 0.2	80	32	34	0
B. cereus (Roma 709)	NCD	10 ± 0.1	12 ± 0.1	40	22	23	0
L. monocytogenes (ATCC 35152)	NCD	NCD	NCD	NCD	20	21	0
S. aureus (ATCC 29213)	12 ± 0.1	15 ± 0.1	15 ± 0.1	40	24	25	0
E. faecalis (ATCC 29212)	NCD	NCD	NCD	NCD	20	12	0
B. subtilis (ATCC 6633)	NCD	10 ± 0.1	15 ± 0.1	60	25	24	0
S. epidermidis (ATCC 12228)	5 ± 0.1	10 ± 0.1	10 ± 0.1	40	18	18	0
S. boulardii (ATCC MYA-796)	NCD	10 ± 0.3	15 ± 0.3	40	0	0	25
C. albicans (ATCC 90029)	NCD	NCD	5 ± 0.1	20	0	0	26
C. tropicalis (M007)	10 ± 0.3	10 ± 0.3	15 ± 0.3	40	0	0	24
C. parapsilosis (M006)	20 ± 0.1	20 ± 0.1	25 ± 0.1	80	0	0	28
C. parapsilosis (ATCC 22019)	NCD	5 ± 0.1	10 ± 0.3	20	0	0	20
C. glabrata (ATCC 15126)	10 ± 0.3	10 ± 0.3	10 ± 0.3	20	0	0	22
Negative control	0	0	0	0	0	0	0

Data are given as mean ± standard deviation of triplicates. Mean values, $n = 3$

NCD no culturable cells detected, *negative control* distilled water, *MIC* minimum inhibitory concentration

[a]Ampicillin (AMP) 10 mcg; [b]gentamicin (GEN) 10 mcg; [c]nystatin (NS) 50 mcg

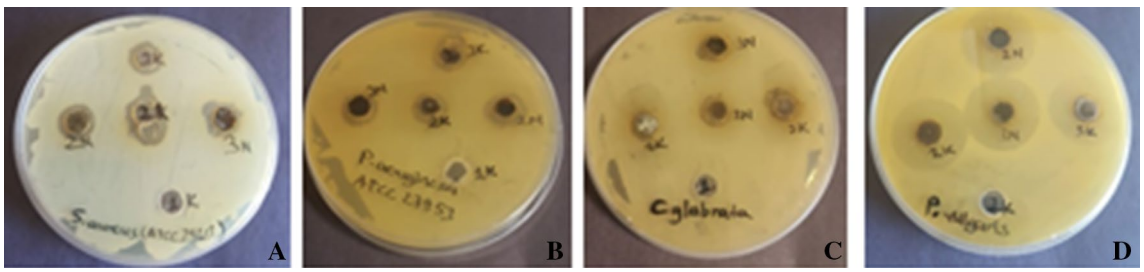

Fig. 4 Inhibition zone of some pathogen microorganisms (**a** *Staphylococcus aureus* ATCC 29213; **b** *Pseudomonas aeruginosa* ATCC 27853; **c** *Candida glabrata* ATCC 15126; **d** *Proteus vulgaris* ATCC 29905) of AgNPs

observed in *K. pneumoniae* (DIZ = 17 ± 0.1 mm), *C. albicans* (DIZ = 5 ± 0.1 mm) and *A. hydrophila* (DIZ = 5 ± 0.1) at a 3 mM concentration level, except for *P. vulgaris*, which exhibited the highest DIZ score at any AgNP concentration (DIZ = 30 ± 0.2 mm). The MIC values are determined, and the results are given in Table 3. MIC values found in this study were accepted as appropriate concentration. The 20 µl concentration of AgNPs inhibited *A. hydrophila* (3 mM), *C. albicans* (3 mM), *C. parapsilosis* (2 and 3 mM) and *C. glabrata* (1, 2 and 3 mM). The antibacterial effect of AgNPs was compared with commercial antibiotics for positive control, and the results of this comparison are given in Table 4 as antimicrobial index. Based on the obtained index data, it

is thought that AgNPs obtained from *P. boryanum* can be considered as an alternative option to today's antibiotics.

Our results may be due to differences in various cell wall configurations and features of the pathogen microorganisms included in the study. The principle antimicrobial effect of silver ions is explained by their high tendency to ionization and dissolution in solutions. Small AgNPs with a higher surface-to-volume ratio exhibit more efficient antibacterial activity

Table 4 (AgNPs: $AgN0_3$ + *P. boryanum*) antimicrobial index

Human pathogen microorganisms	Antibiotics	Antimicrobial index in percentage		
		1 mM AgNP	2 mM AgNP	3 mM AgNP
P. mirabilis (ATCC 29906)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
P. aeruginosa (ATCC 27853)	AMP	NCD	80	80
	GEN	NCD	76.9	76.9
E. aerogenes (ATCC 51342)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
S. typhimurium (ATCC 14028)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
S. dysenteriae (ATCC 11835)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
E. coli (ATCC 25924)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
E. coli (ATCC 25922)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
A. hydrophila (ATCC 7966)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	25
K. pneumoniae (ATCC 21541)	AMP	NCD	NCD	68
	GEN	NCD	NCD	62.9
S. marcescens (ATCC 13880)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
V. anguillarum (ATCC 43312)	AMP	NCD	60	75
	GEN	NCD	54	68
P. vulgaris (ATCC 29905)	AMP	NCD	93	88
	GEN	NCD	93	88
B. cereus (Roma 709)	AMP	NCD	43	54
	GEN	NCD	43	52
L. monocytogenes (ATCC 35152)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
S. aureus (ATCC 29213)	AMP	50	62	62
	GEN	48	60	60
E. faecalis (ATCC 29212)	AMP	NCD	NCD	NCD
	GEN	NCD	NCD	NCD
B. subtilis (ATCC 6633)	AMP	NCD	40	60
	GEN	NCD	41	62
S. epidermidis (ATCC 12228)	AMP	27	55	55
	GEN	27	55	55
S. boulardii (ATCC MYA-796)	NS	NCD	40	60
C. albicans (ATCC 90029)	NS	NCD	NCD	19
C. tropicalis (M007)	NS	41	41	62
C. parapsilosis (M006)	NS	71	71	89
C. parapsilosis (ATCC 22019)	NS	NCD	25	50
C. glabrata (ATCC 15126)	NS	45	45	45

NCD no culturable cells detected, *negative control* distilled water

[a]Ampicillin (AMP) 10 mcg; [b]gentamicin (GEN) 10 mcg; [c]nystatin (NS) 50 mcg

than larger particles. When silver compounds are exposed to intra- or extracellular fluids, they exhibit very high rates of dissolution and ionization (Furno et al. 2004). These ionized silver ions have devastating effects on bacterial cell walls and cellular structures. They tend to accumulate in pathogen microorganisms and form holes in their bacterial cell walls, which eventually lead to their death. Silver ions also have a tendency to bind with microorganism DNA and RNA, making it difficult to synthesize the proteins necessary for reproduction and survival (Sondi and Salopek-Sondi 2004; Morones et al. 2005; Salari et al. 2016). It may be speculated that this effect will be more lethal in organisms with thinner cell walls, while those with thicker cell walls might survive these antimicrobiological effects. This hypothesis is supported by our finding that the number of resistant microorganisms decreased as AgNP concentrations increased. The cell walls of Gram-negative bacteria are also thinner than those of Gram-positive bacteria, which make them more susceptible to the effects of AgNPs even at low concentrations (Sudha et al. 2013).

Our results are compatible with those of several other studies. Biosynthesized AgNPs from green seaweed have been reported to demonstrate good antibacterial activity against many clinical pathogens (Raja et al. 2012). Ramakritinan et al. (2013) also investigated the antibacterial effect of AgNPs biosynthesized from the marine red algae *Gracilaria* sp. The biosynthesized AgNPs exhibited a high level of antibacterial activity against *K. pneumoniae* (DIZ = 6 mm) and *S. aureus* (DIZ = 12 mm). AgNPs biosynthesized from the marine microalgae *C. salina, I. galbana* and *T. gracilis* demonstrated a high level of antimicrobiological activity against *Proteus vulgaris, P. aeruginosa* and *E. coli* (Merin et al. 2010). Hafez and Kabeil (2013) have been tested the antifungal activities of AgNPs synthesized from two microalgae *Chroococcus dispersus* and *Chlorella vulgaris* plant pathogen fungi (*Fusarium solani, Fusarium oxysporium, Helminthosporium* sp., *Sclerotinia sclerotiorum*) and the plant pathogen bacteria (*Pseudomonas flavescens*, *Agrobacterium tumefaciens* and *Erwinia amylovora*). Their results showed that high bacterial and fungal activity was achieved by the resultant nanosilver against the bacteria and fungi investigated. In another study, *Spirogyra varians* was used for the synthesis of AgNPs which an excellent antibacterial effect against *S. aureus*, *L. monocytogenes* and *E. coli* (Salari et al. 2016). Another study on the biosynthesis of AgNPs used the seaweed *Enteromorpha flexuosa* (Wulfen) J. The synthesized nanoparticles exhibited high in vitro antibacterial activity against Gram-positive bacteria and low activity against Gram-negative bacteria (Yousefzad et al. 2014). Patel et al. (2015) examined ability to biosynthesize AgNPs in six cyanobacteria (*Anabaena* sp., *Aphanizomenon* sp., *Lyngbya* sp., *Synechococcus* sp., *Synechocystis* sp. and *Cylindrospermopsis* sp.) and two green algae species (*Botryococcus* sp. and *Coelastrum* sp.). These AgNPs demonstrated effective microbiological effects against *B. megaterium, E. coli, B. subtilis, M. luteus, P. aeruginosa* and *S. aureus*.

Conclusion

AgNPs from the green microalgae *P. boryanum* were successfully synthesized in this study. Nanoparticle characterization was performed using visual confirmation, UV–Vis spectroscopy and FTIR analyses. The algal biomass was exposed to 1 mM, 2 mM and 3 mM silver nitrate ions, and discoloration from bright green to brown was observed, confirming the biosynthesis of AgNPs. Synthesized nanoparticles exhibited a surface plasmon resonance peak at 417 nm. FTIR absorption spectra from cultures possessed 10 clear bands over the wave range of 4000–500 cm^{-1}. These bands were tentatively identified on the basis of reference standards, again indicating the presence of AgNPs. The antimicrobial potential of AgNPs synthesized from microalgae *P. boryanum* was tested against pathogen microorganisms using a well diffusion assay and showed a strong antimicrobial potential. Our results obtained from this study not only confirm the formation of nanoparticles but also show the effective antimicrobial properties of AgNPs. As a result, it is thought that AgNPs can be used as an alternative to commercial antibiotics and also in vivo studies are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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